Development of a chemiluminescence enzyme immunoassay for the detection of aflatoxin M_1

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Abstract. An indirect competitive chemiluminescence enzyme immunoassay (CLEIA) for detecting aflatoxin M_1 (AFM_1) was developed. In the experimentation, antigen coated concentration, antibody dilution proportion, pH value and ionic concentration of the buffer were optimized to obtain the best reaction condition. Finally, the 50% inhibitory concentration (IC_{50}) of the optimized CLEIA is 0.11ng/mL for skimmed milk powder, and the recovery ranged from 77.98% to 104.39%.

Introduction

Aflatoxins are highly toxic mycotoxins which is formed when ruminants ingest feed contaminated with aflatoxin B_1[1]. Currently available research results demonstrate that aflatoxin M_1 can also be present in a wide range of milk-derived products or products containing milk, such as cheese, yogurt, cream and chocolate[2,3].

Several analytical methods are currently available for aflatoxin M_1 determination, including high-performance liquid chromatography associated with fluorescence or mass spectrometric detection [4,5]. Enzyme-linked immunosorbent (ELISA) methods have also been described [6] and are widely employed as screening methods in routine analysis, mainly because of their simplicity and rapidity.

To effectively monitor the occurrence of aflatoxin M_1 in food at low contamination levels, sensitive, reliable and simple analytical methods are required. This work describes the establishment of a sensitive quantitative CLEIA (chemiluminescence enzyme immunoassay). CLEIA is a combination of chemiluminescence (CL) and enzyme immunoassay, it has combined advantages of the high sensitivity of CL and the high specificity of immunoassay[7].

Materials and methods

Reagents and instrument: Mouse anti-aflatoxin M_1 monoclonal antibody was obtained from our own laboratory. Horseradish peroxidase-labeled goat anti-mouse IgG-HRP were purchased from sigma (St. Louis, MO, USA). Chemiluminescence substrate was purchased from Helisence (Shanghai, China). Phosphate-buffered saline (PBS), PBS with 0.05% Tween-20 (PBST), Well wash versa (Thermo scientific, USA), 96-well white polystyrene plates (Costar, USA), 5804R High-speed Refrigerated Centrifuge (Eppendorf, Germany).

Determination of AFM_1 by CLEIA: CLEIA was carried out using 96-wells white polystyrene plates. The plates were coated by adding into each well 120μL of AFM_1-BSA antigen dissolved in PBS, and incubated at 37℃ lucifuge moisturize for coating 2.5h, then washed with PBST and blocked by adding 330μL per well of 5% skim milk powder, which incubated at 37℃for 3.5h. After washing 3 times with PBST, 100μL of horseradish peroxidase (HRP)-conjugated anti-antibody were added to each well incubating condition as the former except for time (45min). Finally, 100μL substrate solution prepared freshly was added into each well. The chemiluminescence intensity was

monitored on a Luminoskan Ascent (Thermo, USA). In the experiment, the RLU_{max}/IC_{50} ratio was used as a parameter to judge the impact of factors.

Sample Preparation

The 1g of skimmed milk powder sample was suspended in 10 mL of 50 °C distilled water. 20ml acetonitrile was added to extraction the spiked aflatoxin M_1, and the mixture was shaken on a rotary shaker for 10 min. The extracting solution was centrifuged at 6000 r/min for 10 min at 4°C. The supernatant was obtained and heated in water bath to evaporating off the acetonitrile. Then the sample was diluted with water.

Result and discussion

Optimized concentrations of coating antigen

The concentrations of the coating antigen was optimized by the phalanx titration which the antigen coated concentration range from 0.25μg/mL to 4μg/mL, and the antibody dilution proportion range from 1:80000 to 1:5120000. The result was showed in Fig.1, AFM_1-BSA conjugate concentration of 0.5μg/mL is optimum for coating the plates.

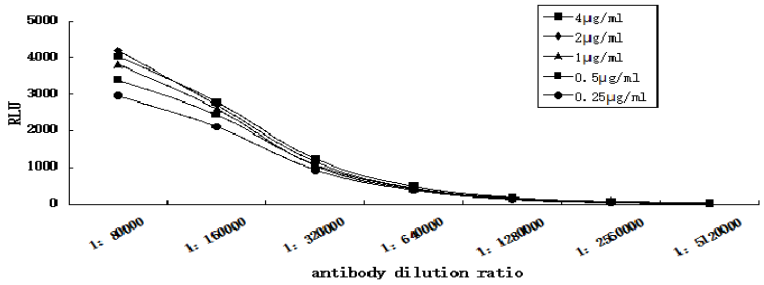

Fig.1. The RLU effect of varied concentrations of coating antigen

Optimized the antibody dilution proportion

The antibody dilution proportion was optimized by the indirect competitive. The certified reference material was diluted with purified water to 0.02ng/mL, 0.05ng/mL, 0.2ng/mL, 1ng/mL, 2.5ng/mL and doubling dilution for antibody concentration range from 1:320000 to 1:2560000. From the Fig.2, the best dilution proportion of antibody is 1:320000.

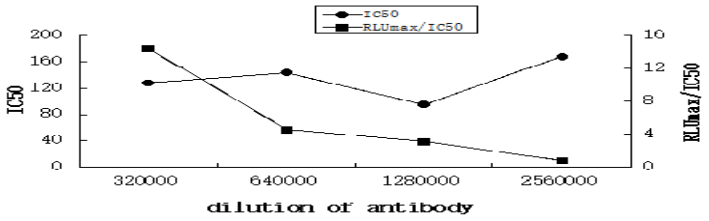

Fig.2. The effect of antibody dilution proportion

Optimized the ionic strength and the pH value of the buffer

In this step, the ionic strength of the buffer ranges from 0 mol/mL to 2 mol/mL, the pH value of the buffer ranges from 5.5 to 9.0. The result showed that the pH value of buffer showed in Fig.3 (b) is ruleless to luminescence value. So the optimum condition of ionic strength and pH value is 1mol/L、7.0, respectively in the Fig.3(a) and Fig.3 (b).

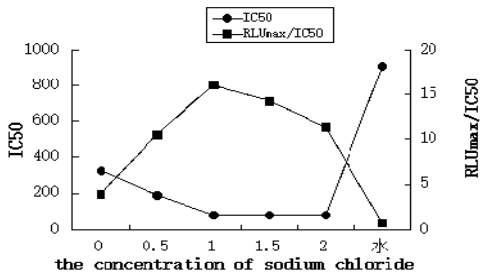

Fig.3(a). The effect of sodium chloride

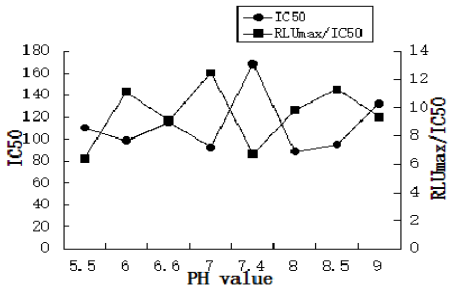

Fig.3(b). The effect of the pH value

Optimized the concentration of organic solvents

The certified reference material of AFM_1 was diluted different concentrations of methanol and the purified water. From the Fig.4, the best result is with purified water dilution.

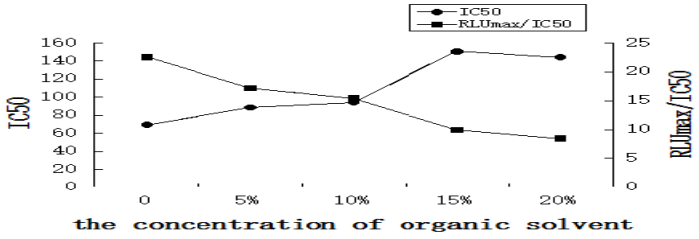

Fig.4. The effect of the concentration of organic solvent

Establishment of the Standard Curve

In the assay, the later step experiment condition used the result of the former. According to the optimized parameters, the standard curve is shown in Fig.5. The horizontal coordinate in the Fig.5 was obtained by enlarging 1000 times of original concentration then calculating the logarithm. The IC_{50} was 0.11ng/mL. The linear working range determined was 0.05-0.4ng/mL.

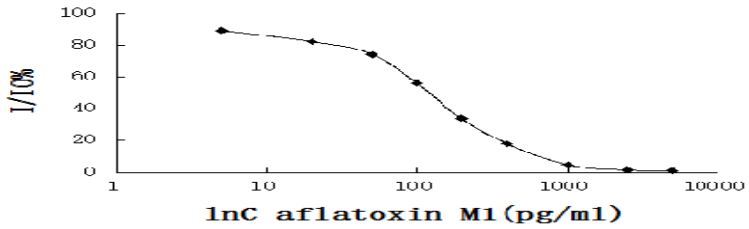

Fig.5. The inhibition curve of aflatoxin M_1

Recovery Studies of Spiked Samples

In this procedure, we take action of diluting to eliminate the interference. The recovery ratio and SD value for spiked skimmed milk powder are listed in table 1.

The table 1 shows that the recovery ratio of the experiments are acceptable and the all SD values are lower than 10%. It proves that the CLEIA method has a good precision.

Table 1 The recovery ratio and SD value for skimmed milk powder

Adding standard	Recovery ratio	SD value
10ng	93.48%	2.53%
20ng	104.39%	8.94%
30ng	77.98%	2.55%

Conclusion

In the experiment, an action of diluting was exploited to eliminate the sample matrix interference. A sensitive and efficient CLEIA has been established to detect aflatoxin M_1. This method was performed with wide linear range and low LOD for skimmed milk powder. The recoveries ranged from 77.98% to 104.39%. The method has showed a good precision for aflatoxin M_1 detection and it is suitable for detection of trace aflatoxin M_1 in food.

Acknowledgement

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